Phytoplankton Imaging and Analysis System: Instrumentation for Field and Laboratory Acquisition, Analysis and WWW/LAN-Based Sharing of Marine Phytoplankton Data (DURIP)

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Award Number: N00014-99-1-0598
http://thalassa.gso.uri.edu/pial
http://thalassa.gso.uri.edu/ESphyto
http://thalassa.gso.uri.edu/rines
http://www.gso.uri.edu/criticalscales

LONG TERM GOALS

Our long term goal is to understand the ecology of phytoplankton, especially the large, colonial diatoms which frequently dominate the flora of coastal shelves, upwelling areas, fjords and banks. We are interested in ways in which species-specific properties, including cell and colony size and shape interact with physical mixing processes to regulate the spatio-temporal distribution of diatoms. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, size structure, and the impact of species-specific characteristics of the phytoplankton on ocean optics.

OBJECTIVES

This award addresses that goal by providing instrumentation and computer systems which facilitate the processes of: (1) acquiring images of the different kinds of plankton which are present in our samples, (2) electronically sharing those images (data) via the world-wide-web (WWW), or local networks (LAN), (3) quantifying size, shape, and other parameters of plankton cells and colonies via image analysis and image reconstruction, and (4) creating educational materials (e.g. lectures, videos etc.).

APPROACH

The components of the Plankton Imaging and Analysis Laboratory (PIAL) have been selected to provide us with a series of new capabilities for both laboratory and field work:

(1) Microscopes. Our microscopy facilities combine the best offerings of the Nikon and Zeiss instrument lines. The Nikon system is equipped with phase contrast, brightfield, Nomarski Differential Interference Contrast and Epifluorescent optics, primarily for use in the lab. Nikon provides superb ergonomics, and ease of use for novice users (students). However, while Nikon's phase contrast is commendable in black and white, it is significantly compromised in color. Carl Zeiss provides what I believe to be the best phase contrast optics commercially available. The Zeiss microscope is equipped with phase contrast, and limited Nomarski

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEP 2000		**00-00-2000 to 00-00-2000**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Phytoplankton Imaging and Analysis System: Instrumentation for Field and Laboratory Acquisition, Analysis and WWW/LAN-Based Sharing of Marine Phytoplankton Data (DURIP)	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Graduate School of Oceanography, University of Rhode Island,,South Ferry Road,,Narragansett,,RI,02882**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**6**	

capabilities. It will be used extensively in the field. The Zeiss Akioskop 2 is also equipped with a motorized focus mechanism, which can be interfaced with a computer to allow optical serial sectioning of specimens. A "z-stack" of serial sections can be recombined to create a single, in-focus image of a highly three-dimensional specimen. An extension of this approach (which requires additional, sophisticated software packages) can compose full, 3-D interactive reconstructions of specimens.

(2) Digital documentation of plankton. Standard, film-based imaging requires exposure of the film (usually an entire roll), commercial processing (for color film), and subsequent scanning of prints or slides into the computer. Digital imaging systems bypass most of these steps, and allow direct processing of single, or multiple images. Much time is saved, because all stages of the work take place in our own facilities. The Optronics camera also allows low light imaging, and can be used for epifluorescence photomicrography, and photography of bioluminescent specimens.

(3) Video. Many phytoplankton (and most zooplankton) are motile. It is not possible to attain an appreciation of the effect this feature can have on their life-histories, and spatio-temporal distribution in the sea from still images. The Optronics video systems allow us to record live, video images of motile taxa in standard S-video, RGB or digital format. Adobe Premiere software can be used to produce illustrative or educational clips, which can be distributed via CD, videotape, or via the web.

(4) Film-based imaging. Digital imaging offers tremendous advantages for flexibility and speed of processing, but there remain applications for which the superior resolution of film is the best medium. For this reason, the system includes Nikon's automatic 35 mm camera system, which will allow us to take high resolution photographs when needed to answer specific questions.

(5) Image Analysis. Current capabilities include Zeiss' Axiovision (with the measurement module), and NIH Image.

(6) Computers. The facility uses both MacIntosh and PC platforms. Zeiss computerized microscope software and imaging runs on a Dell Pentium III, 600 mHz machine. Sony digital video tape recording equipment runs into a Power Macintosh G4, via a Firewire connection. A Dell Inspiron 7500 can be used in the field.

(7) Communication/data sharing. A variety of hardware and software systems streamline the process of acquiring images, and placing them on the web. In future field experiments, we will be able to utilize a wireless network, such as the one implemented by Cowles in East Sound (1998), to relay images of plankton to our colleagues, to a classroom, or to interested parties in other locations in near-real time.

Further information on specific components which make up this facility, and on their technical capabilities can be found on the PIAL web site (http://thalassa.gso.uri.edu/pial).

WORK COMPLETED

The Plankton Imaging and Analysis Laboratory (PIAL) consists of numerous components, which function together to provide new capabilities to our research team. These include microscopes, computer systems, digital and 35 mm camera systems, digital video recording equipment and image processing, image analysis, digital video editing and web authoring software. The majority of instrumentation has been in place for quite some time, but we continue to add components that improve the system. For example, Optronics has recently provided us with a custom camera adaptation. With it,

we can image much larger particles/diatom colonies on the compound microscopes. I have also been able to attach it to a Zeiss SV-8 stereo microscope (owned by Donaghay). I purchased a special, photographic objective for this instrument. We can now acquire extremely high quality still images and video of zooplankton, complementing our phytoplankton work.

RESULTS

We are using our new equipment in conjunction with several projects: (1) Laboratory experiments on the effects of small-scale turbulence on the morphology of colonial diatoms. These experiments are designed to aid in the interpretation of our field data from East Sound. They will be conducted in conjunction with instrument tests carried out under Donaghay & Dekshenieks' NOPP project. PIAL equipment is being used for image acquisition and analysis of phytoplankton from the experimental tanks. (2) I used the digital video acquisition and editing capabilities of the system to make a 20 minute movie of zooplankton and protists, used in the "Dazzling Diversity" presentation (below). This approach will be used in the future to produce scientific and educational multimedia presentations. (3) The instrumentation has been used to acquire web-ready still images. I have used the digital video editing software to make prototype streaming QuickTime movies of plankton, which can be viewed on the web: (http://www.gso.uri.edu/~jrines/movietest.html). I intend to further develop this protocol, and make a web-based library of movies of planktonic organisms.

PIAL instrumentation has also been used by colleagues (described under Related Projects, below).

IMPACT/APPLICATIONS

In addition to expanding our oceanographic research capabilities, this equipment serves an important educational function because it facilitates the sharing of visual, species-specific information about plankton with several different audiences. Familiarity with phytoplankton at the species-specific level has traditionally been limited to taxonomists and ecologists. It is usually published in a highly specialized literature, which is read mainly by other taxonomists. However, I have found that researchers in many other areas are very interested in the properties of phytoplankton when they can easily access the information. This is best accomplished using a direct, visual approach — pictures. Use of the web as a vehicle for dissemination of information allows anyone who is interested to find it via search engines.

The Zeiss Axioskop-2 is capable of computer-controlled, optical serial sectioning of plankton specimens. This capability allows for the future development of 3-D reconstruction techniques that will allow us to visualize microscopic plankton in 3-D, and render the object/shape on the computer. Quantitative data describing the 3-D shape of an organism may allow us to interact with both optical oceanographers, and Navy systems developers in new ways.

TRANSITIONS

This equipment will facilitate the development of the plankton web sites developed in conjunction with ONR and NSF sponsored research. These sites have proven very useful in distributing information on plankton to a large and diverse community of interested people, including academic research scientists,

high school and college students, journalists, environmental monitoring personnel, government agencies, aquaculture companies and the general public.

RELATED PROJECTS

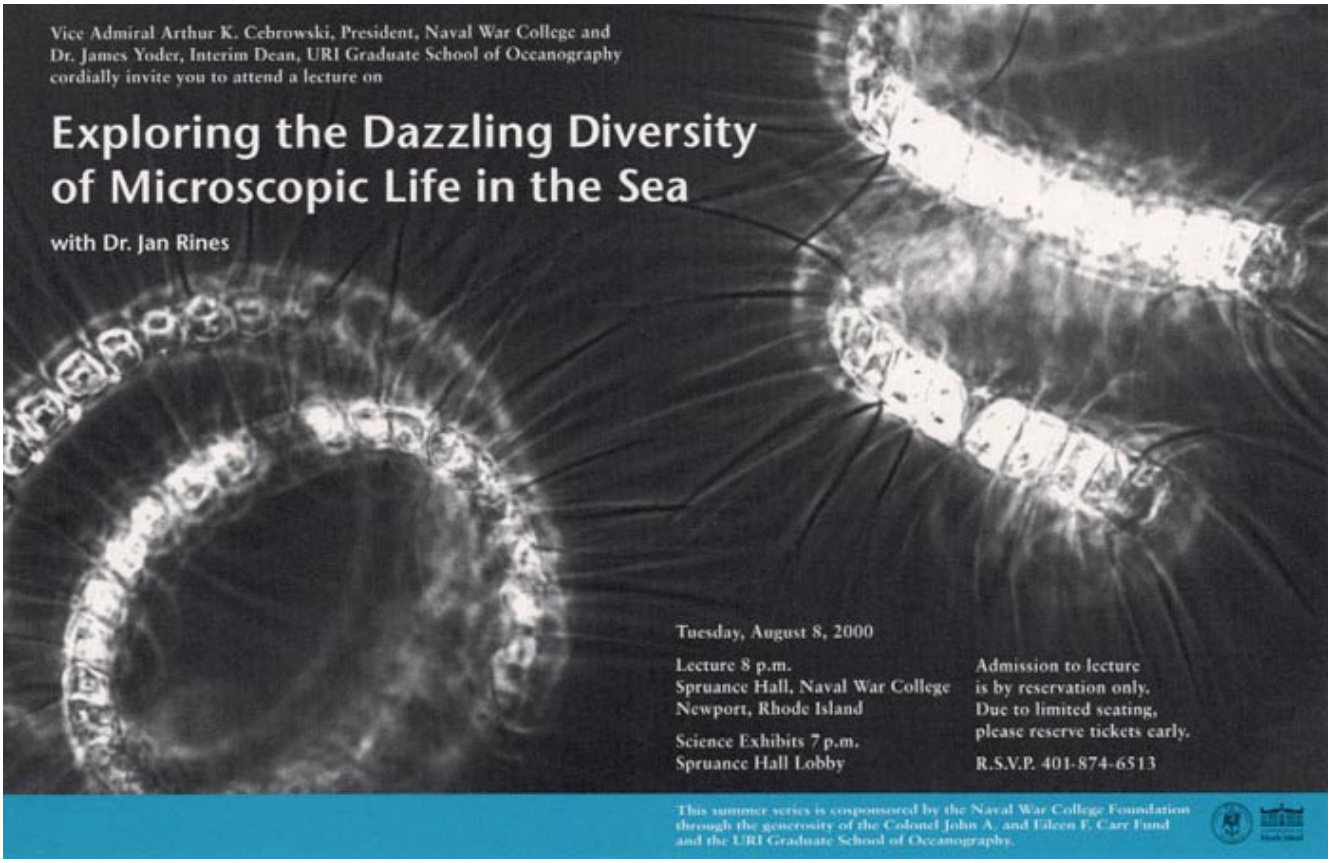
This equipment provides us with capabilities which will allow us to interact with other efforts. For example, in future field efforts, this equipment will allow us to utilize a wireless network such as that constructed by Cowles (DURIP N00014-97-1-0349) to rapidly share visual information on phytoplankton. It will facilitate our interaction with other Thin Layers PIs who are interested in species-specific information, for example Donaghay, Holliday, Twardowski, Weidemann, Zaneveld, Alldredge, Perry, Cowles, Gifford.

This equipment was used by James M. Sullivan (GSO/URI) to make digital photographic plates illustrating intracellular movement of chloroplasts and scintillons in bioluminescent dinoflagellates. This work was included in his PhD dissertation.

The equipment was also used by Jennifer Crain, a student at Oregon State University, while she worked with colleagues at GSO/URI. Their study looked at the relationship between the growth and cellular activity of planktonic copepods with respect to food availability. It examined the relationship between RNA/DNA ratios and the molt cycle in *Calanus finmarchicus.* Intermolt development was determined using jaw phases, following methods outlined by Miller et al. (1990). In order to see the jaw phases, a jaw was dissected from each individual copepod and examined using my Nikon Microscope, equipped with differential interference contrast optics. Digital photographs taken using my equipment were used in a manuscript submitted to Deep Sea Research (Crain, J.A. & C.B. Miller – Effects of starvation on intermolt development in *Calanus finmarchicus* copepodites: a comparison between theoretical models and field studies).

PRESENTATION

I was the speaker for this years' Annual Summer Lecture, a series co-hosted by the Naval War College, Newport, R.I. and the Graduate School of Oceanography, URI. Over 500 people were in attendance. I used my new DURIP microscopy, video and digital imaging equipment to prepare this presentation. In addition to color slides, I used about 20 minutes of digital video of microscopic, planktonic life in the sea.



The full description of this talk is available on-line: http://thalassa.gso.uri.edu/rines